

## DATA SHEET

### Antigen Retrieval Citra (pH-6.0)

Cat. Nos. HK086-5K, HK086-9K, HK086-XAK, HK087-5K, & HK087-20K

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Doc. No. 932-HK086, Rev. No. G  
Release Date: 18-Jul-2024

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#### REAGENTS SUPPLIED (Store at 2-8°C)

One of the following:

HK086-5K:	One bottle (100 ml), 10x Concentrated, Antigen Retrieval CitraSolution
HK086-9K:	One bottle (500 ml), 10x Concentrated, Antigen Retrieval Citra Solution
HK086-XAK:	One bottle (1000 ml), 10x Concentrated, Antigen Retrieval Citra Solution
HK087-5K:	One bottle (250 ml), ready-to-use, Antigen Retrieval Citra Solution
HK087-20K:	One bottle (1000 ml), ready-to-use, Antigen Retrieval Citra Solution

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#### Intended Use

This product may be used to recover antigenicity in formalin-fixed, paraffin-embedded tissue. This product is for laboratory use only.

#### Description

The Antigen Retrieval technique is a novel method for the rescue of antigens from formalin-fixed, paraffin-embedded tissue. It consists of heating sections in a microwave oven in the presence of an antigen retrieval solution.

The quality of the staining result is largely dependent on **strict adherence** to the antigen retrieval protocol. If the antigens are incompletely retrieved, the staining is light and the background may be high. A user following the standard protocol outlined on page 2 will see crisp, clear staining with little or no background.

#### Limitations

The antigen retrieval protocol is recommended for use with tissues fixed *with formalin only*. Other fixatives or fixation procedures may not produce comparable results. Interpretation of the staining results is solely the responsibility of the user.

#### Concentrate

For concentrate, dilute antigen retrieval with deionized water in a 1:10 ratio before use.

#### Standard Protocol

1. After dewax and rehydration, Rinse slides in deionized water. Place slides in a plastic staining holder\*\* with any empty slots filled with blank slides. Place the holder in a slide bath containing 250 ml of Antigen Retrieval CitraSolution. Place the lid loosely on the bath and center it inside a microwave oven on a paper towel to adsorb any liquid runover.
2. Turn the oven on high power (500-1,000 watts) and closely watch the solution until it comes **to a rapid boil**, and then turn off the oven. (Note: It usually takes 3-7 minutes before a boil is reached. However, the amount of time required may vary significantly depending on a number of factors, such as the starting temperature of the retrieval solution, the wattage of the microwave oven, the age of the oven and the inside temperature of the oven. It is very important that a rapid boil is reached for every run before proceeding to the next step.)
3. Set oven power to approximately 50% level and heat for 10 to 15 minutes. (Note: The power setting should be adjusted so that the oven cycles on and off every 20-30 seconds and the solution boils about 5-10 seconds each cycle. A

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successful run should give frequent, but not continuous boiling without any liquid runover. This power setting should be noted and used for this step in all subsequent runs for the same antibody. Each antibody should be tested for the optimal time for this step.)

4. Remove the slide bath from the microwave oven. Allow slides to cool for 20-30 minutes at room temperature. Rinse with several changes of deionized water. Place slides in 1X PBS and continue with the immunostaining procedure.

### Alternate Method: Microwave Pressure Cooker Protocol

The alternate method uses a pressure cooker inside of the microwave. The pressure cooker that we recommend is available through Nordic Ware<sup>®</sup>, or you may purchase it through BioGenex for your convenience\* .

1. Place slides in a slide holder\*\* filling empty slots with blank slides. Put the holder in the white slide bath containing 250 ml of working strength Antigen Retrieval Citra Solution. Put the lid loosely on the bath (placed at an angle to permit release of pressure).
2. Fill a plastic pressure cooker with 600 ml of distilled water. Place up to three baths side-by-side in the pressure cooker and cover the pressure cooker according to the manufacturer's instructions.
3. Center the pressure cooker inside the microwave oven, and turn the oven on high power (800-850 watts) for about 15 minutes, until the cooker is fully pressurized.
4. Reduce the microwave power level to 300-350 watts (40% power for 800 watt ovens) and allow the solution to simmer for 15 minutes. Then remove the pressure cooker from the microwave oven and carefully open the cover according to the manufacturer's instructions. Leaving the slides in the cooker, allow the slides to cool for 20 minutes, and then continue with the immunostaining procedure.

### References

1. Shi, S.R., et al. Antigen Retrieval in formalin-fixed, paraffin-embedded tissues: an enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections. *J HistochemCytochem* 39:741, 1991.
2. Gown, A.M., et al. Microwave-based antigenic unmasking: a revolutionary new technique for routine immunohistochemistry. *ApplImmunohistochem* 1:256-266, 1993.
3. Shi, S.R., et al. Antigen Retrieval technique: a novel approach to immunohistochemistry on routinely processed tissue sections. *Cell Vision* 2:6-22, 1995.
4. Shi, S.R., et al. Antigen Retrieval immunohistochemistry under the influence of pH using monoclonal antibodies. *J HistochemCytochem* 43:193-201, 1995.

†This product is covered by one or a combination of the following U.S. patents and their foreign equivalents:  
U.S. Patent No. 5,244,787 & 5,578,452.

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\* *Microwave Tender Cooker*<sup>®</sup>, Cat. No. 62104, Nordic Ware<sup>®</sup>, Minneapolis, MN; BioGenex Cat. No. NWPlus001-PC.

\*\* *The microwave Antigen Retrieval accessory kit (MW001-HB) contains 3 sets of slide baths, lids and holders. These are similar to the Tissue Tek*<sup>®</sup> *gray slide holders for 22 slides and the WHITE staining dish with lid which will also work well with these protocols.*

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